PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P26480PC00/TWI	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/12794	International filing date *(day/month/year)* 07.11.2003	Priority date *(day/month/year)* 08.11.2002
International Patent Classification (IPC) or both national classification and IPC C12N9/96		
Applicant AVANTIUM INTERNATIONAL B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
28.05.2004	17.02.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Mauhin, V Telephone No. +49 89 2399-7027

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/12794

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-19 as originally filed

Claims, Numbers

1-15 as originally filed

Drawings, Sheets

1/1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-9,11-13
	No:	Claims	1,2,10,14,15
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-15
Industrial applicability (IA)	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following document (D) is referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: LOPEZ-SERRANO P ET AL: 'Cross-linked enzyme aggregates with enhanced activity: Application to lipases.' BIOTECHNOLOGY LETTERS, vol. 24, no. 16, August 2002 (2002-08), pages 1379-1383, XP009003377 August, 2002 ISSN: 0141-5492

1. The present application relates to a method for the preparation of crosslinked enzyme aggregates (CLEAs) comprising enzyme molecules being associated with an enzyme-activity enhancing agent interacting with said enzyme molecules. Said enzyme can be cofactor dependent like an alcohol dehydrogenase. Said cofactor can be NAD+, NADH, NADP+, NADPH or an analogue of said nicotinamide cofactors. The method comprises the steps of aggregating a plurality of enzyme molecules with a precipitating agent in a liquid medium and crosslinking the aggregates obtained with a crosslinking agent in a liquid medium; during or between said steps, en enzyme activity-enhancing agent is added and allowed to become and remain associated with the enzyme molecules. The crosslinking agent can be prepared by combining two compounds, each having at least two reactive groups, primary amino groups for the first compound, aldehyde groups for the second compound. At least two different cofactor dependent enzymes can be provided with their respective enzyme activity enhancing agents. A further step of recycling the CLEAs after the performance of an enzyme (CLEAs) catalysed reaction can be added. Said recycling can be performed simultaneously with the reaction when the CLEAs comprise at least two cofactor dependent enzymes, the first enzyme being associated with the cofactor in oxidised form and the second one being active in catalysing a second reaction when associated with said cofactor in a reduced state. Crosslinked enzyme aggregates comprising an enzyme activity enhancing agent associated therewith are also disclosed.

2. Document D1 refers to CLEAs with enhanced activity, wherein said enzyme is a lipase (i.e. a cofactor dependent enzyme). CLEAs were prepared by the steps of precipitating said lipase molecules with a precipitating agent and crosslinking the obtained aggregates, in the presence of additives which enhance the activity of the

enzyme (i.e. enzyme activity enhancing agents). Both steps were simultaneously performed (page 1380, "CLEAs prepared in the presence of SDS", "CLEAs prepared in the presence of Triton X-100", "CLEAs prepared in the presence of dibenzo-18-crown-6"; Figure 1; page 1382, "Preparation of CLEAs": first paragraph, "Effect of additives": first three paragraphs). Said additives were eventually washed out of the CLEAs (page 1382, "Effect of additives": second paragraph).
In view of the drafting of present claim 1, it appears that the method disclosed in D1 encompasses the method of claim 1. Indeed, if one stops the preparation of the CLEAs of D1 before the step of washing out the additive, one will obtain a CLEA in which the enzyme molecules are associated with an enzyme activity-enhancing agent (interacting with it). That means that the CLEAs obtained by the method of claim 1 are intermediate products of the method used in D1.

Therefore, the subject-matter of claims 1, 2, 10, 14 and 15 is anticipated by the disclosure of D1 (Article 33(2) PCT).

Claims 3-9 and 11-13 meet the requirements of Article 33(2) PCT.

3. Interestingly, the Applicant gives a definition of the term "become associated" in the description (page 4, line 22 - page 5, line 2). Therein, he explains that, in D1, the crown-ether or the surfactant (i.e. the enzyme activity-enhancing agent) has to be removed from the CLEAs in order to render the enzyme active. Thus, one has to understand that it is not possible to use any kind of enzyme activity-enhancing agent in the method of **claims 1 to 3 and 5-15**. Therefore it appears that the problem to be solved by the invention cannot be solved over the whole scope of said claims. In fact, in view of D1, it seems that the effect of one enzyme activity-enhancing agent depends on the type of enzyme (type of lipase) used (see Tables 1 and 2). Again, it seems that the problem to be solved by the invention cannot be solved over the whole scope of **claims 1-6 and 8-15**.

Consequently, claims 1-15 do not involve an inventive step (Article 33(3) PCT).

4. The Examiner would like to drawn the attention of the Applicant that the definition of the term "become associated", i.e. "the enzyme molecule and the enzyme activity-enhancing agent are allowed to remain associated to one another, so that during any enzymatic reaction performed with the CLEAs thus prepared, the enzyme activity-enhancing agent is still allowed to be associated with the enzyme molecules" corresponds to a **result to be achieved** which merely amounts to a statement of the

underlying problem. The **technical features** necessary for achieving this result are not defined. Thereby, claim 1 which relies on said definition is not clear and does not fulfill the requirements of Article 6 PCT.